

**RAPID BACTERIAL TESTING FOR SPACECRAFT WATER****NASA Research Grant NAGW-5001****Report to NASA Headquarters, Washington D.C.****OCTOBER 1996**

by

John T. Lisle, Barry H. Pyle and Gordon A. McFeters

Department of Microbiology, Montana State University  
Bozeman, Montana 59717**PERSONNEL:**

Activity on this project commenced in the spring of 1996 with Dr. McFeters as principal investigator. Dr. Pyle has acted in the role of co-principal investigator, although he will be taking a less active role than anticipated because of other research commitments in the laboratory, although he is available on a day-to-day basis. As a result, it was decided to appoint a post-doctoral research assistant. Advertisements were placed nationally and locally, and John Lisle, who was completing his doctorate in the Public Health program at the University of South Florida, Tampa. Dr. Lisle joined us in June, 1996. Susan Broadaway, Research Associate, undergraduate student assistants Ryan Storfa, Carla Johnsrud, and Scott Burnett, along with undergraduate lab aids (College Work Study) Jennifer Canwright and Kari Farrell (Summer 1996) and Nicole Pilakowsky and Brian VanderVen (Fall 1996 through Spring 1997). Undergraduate student assistants are enrolled for independent study research credits, and are not paid for this work, and the cost of CWS is shared over several projects.

**PROGRESS:**

Progress will be addressed by Task (Research Tasks, pages 7-10 of the original proposal). The majority of time spent on the project to date has been focused on familiarizing the post-doctoral research assistant (Dr. John Lisle) with the techniques developed in this laboratory and modification of those techniques to meet the objectives of the project.

**Task 1. Organism Selection.** *Escherichia coli* O157:H7 is currently being evaluated as the reference strain for the development of Tasks 2-6. This bacterium is routinely used in this laboratory and is well characterized in regard to its growth requirements and characteristics. The other species listed in the proposal are on site and will be evaluated using the protocols developed with *E. coli* O157:H7.

**Task 2. Antibody and Probe Acquisition or Production.** To date the following labeled and unlabeled antibodies have been purchased for use in direct and indirect antibody staining assays, respectively: (direct) anti-*E. coli* O157:H7/FITC labeled, (indirect) anti-*E. coli* O157:H7, anti-*Klebsiella*, anti-*Salmonella* and anti-*Pseudomonas aeruginosa*. The secondary, labeled antibodies used in the indirect assays include the FITC and TRITC fluorochromes. Currently, *E. coli* O157:H7 is being used to optimize antibody staining assays in conjunction with fluorescent probes used to assess physiological status (see Task 6).

Oligonucleotide probes complimentary to 16s rRNA sequences within *Ps. aeruginosa* are available in this laboratory from previous studies. We are currently performing preliminary experiments using these probes to optimize a fluorescent in situ hybridization (FISH) protocol. Upon the development of a optimized, but general, protocol for this technique we will purchase the 16s rRNA probes for other bacteria listed in the proposal.

**Task 3. Antibody and Probe Specificity.** Due to working with pure *E. coli* O157:H7 cultures, to date we have not evaluated the specificity of two or more fluorescently labeled antibody in a consortia of bacteria. However, if we continue to use our current suppliers, we anticipate minimal problems with specificity since determining the degree of cross-reactivity of an antibody with bacteria which may be commonly found associated with the target bacterium is a standard quality assurance assay. If we decide to develop a specific antibody, Montana ImmunoTech, Inc. will be employed to produce the antibodies and we will be responsible for determination of its specificity.

As stated in Task 2, the FISH protocol has not yet been developed. Oligonucleotide probe specificity is central to a reliable FISH assay. The FISH assays we are currently using as general protocols, outline specific factors that must be addressed and optimized to achieve specificity and reproducibility.

**Task 4. Membrane Filtration Optimization.** See Task 6.

**Task 5. Physiological Assessment Methods.** See Task 6.

**Task 6. Combination of Techniques.** Most of the efforts to date have been directed towards the development of a technique that allows the staining of bacteria retained on black polycarbonate filters with two or more fluorochromes. *E. coli* O157:H7 has been selected as a reference strain for development of a general protocol that will be applied and modified if necessary for the other bacterial species listed in the proposal. The objectives of this approach are to determine from a single filter the total number of bacteria present in a water sample, if a specific strain of bacteria is present within the total population and if these bacteria are viable or dead. The different categories and the stains or probes used to determine these criteria include: (1) total cell counts using DAPI and acridine orange, (2) identification of specific strains of bacteria using TRITC and FITC labeled secondary antibodies raised against anti-*E. coli* O157:H7 primary antibodies, (3) detection of respiratory activity using CTC, (4) detection of nutrient responsiveness using the direct viable count (DVC) technique, (5) detection of membrane potential using calcofluor white, DiOC<sub>6</sub>(3) and Rh123, (6) detection of loss of membrane potential using DiBAC (3) and (7) determination of viability using the commercially available Live/Dead BacLight kit (Molecular

Probes, Inc., Eugene, OR). Preliminary studies have been conducted to optimize conditions for each stain or substrate alone and in various combinations. In addition to these assays, agar plate assays for determination of total viable counts (R2A agar) and lethality and injury within populations of bacteria after exposure to disinfection (TLY and TLYD agar) have been incorporated. Calcofluor white and DiOC<sub>6</sub>(3) have demonstrated unpredictable results and will not be evaluated further.

A modified version of the CTC/fluorescent antibody filter assay outlined in Figure 2 of the original proposal has been used with *E. coli* O157:H7 cells. Bacteria retained on black polycarbonate filters were incubated with CTC, stained with TRITC labeled fluorescent antibodies, followed by staining with DiBAC<sub>4</sub>(3). The modified protocol produced promising results. This is encouraging since the ability to evaluate a sample with more than one fluorogenic stain or probe should facilitate the use of laser scanning technologies coupled with image analysis for rapid detection of target bacterial species. Other combinations of stains and substrates from the list previously mentioned are being evaluated in regard to their applicability using this protocol.

We are also continuing research on the use of immunomagnetic separation as a means of concentrating specific bacteria from water and foods. To determine the efficiency of bacterial detection by immunomagnetic separation (IMS) and the compatibility of IMS with cyanoditolyl tetrazolium chloride (CTC) incubation to determine respiratory activity, using the pathogen *Escherichia coli* O157:H7. Staining with DAPI or a specific fluorescein-conjugated anti-O157 antibody was used to allow visualization of bacteria by epifluorescence microscopy. Suspensions were also plated on tryptone lactose yeast extract (TLY) agar. *E. coli* O157:H7 grown in broth were diluted in phosphate buffered saline (PBS) or sterile water and reacted with immunomagnetic beads coated with anti-O157 specific antiserum. Beads were obtained Dynal (Dynabeads), Bangs Laboratories (Bangs), or Milltenyi Biotec (Mini Macs). The largest of these, Dynabeads (2.8 $\mu$ m), were obtained pre-coated with anti-O157 antibody. Bangs beads (ca. 0.6 $\mu$ m) and Mini Macs (ca. 0.05 $\mu$ m) were coated with rabbit anti-O157 serum (Difco).

Initial experiments with Dynal beads achieved recovery rates of ca. 80%, and the presence of the beads did not affect the determination of respiratory activity indicated by CTC reduction. Mini Macs beads recovered more than 94% of the bacteria in a 50ml suspension containing ca. 10<sup>5</sup> CFU/ml of bacteria.

**Task 9. Effects of Growth Conditions and Stressors.** Even though this task has been scheduled for the second year, preliminary studies are being conducted to optimize conditions for evaluation of fluorogenic stains and probes after disinfection or starvation. *E. coli* O157:H7 is being used as the reference strain. Preliminary studies have been completed that evaluated the specificity and reliability of TRITC labeled fluorescent antibodies, CTC, DiBAC<sub>4</sub>(3) and the Live/Dead BacLight kit after *E. coli* O157:H7 cultures were exposed to lethal temperatures and concentrations of formalin, ethanol and chlorine. Equal volumes of the inactivated culture and a viable culture of the same strain were mixed and stained or probed. Cell counts using the listed stains and probes were compared to total cell counts using DAPI and total viable counts using R2A agar. All stains and probes evaluated demonstrated consistent and predictable results under these experimental conditions.

Currently, protocols for the evaluation of sub-lethal concentrations of chlorine are being developed. Sub-lethal injury is being evaluated using the same stains and probes listed above, with the addition of Rh123. Injury is also being determined by use of TLY and TLYD plate agars, where TLYD plates contain deoxycholate which inhibits the growth of injured bacteria. The ratio of (TLY-TLYD)/TLY plate counts gives an estimate of injury in the exposed population. Comparisons of the stain/probe cell counts to the plate counts will provide an indication of the applicability of the listed stains and probes for evaluation of sub-lethal injury. The protocol developed for these types of assays will be used and modified if necessary for the starvation experiments.

**Task 11. Preliminary Automation Assessment.** We have recently upgraded our computer system to make the image analysis software system, Optimas, run more efficiently. Also, we have ordered a ChemScan analysis system using Department of Defence (Army) funding. This system includes a dual channel laser that can excite two different fluorochromes or probes simultaneously. It can scan an entire filter in less than three minutes, locating and counting cells of specific size and fluorescence (i.e., color). We will be incorporating a computerized mechanical microscope stage to confirm all positive cell locations by direct microscopic examination. The ChemScan compliments our primary objective of rapid identification of specific bacteria on a filter.

#### **RESEARCH PLANS:**

1. Evaluations of the fluorogenic stains and probes will continue. *E. coli* O157:H7 will be used as the reference strain for optimizing protocols. We anticipate the continued use of the fluorescent antibodies (TRITC and FITC labeled) in conjunction with CTC, Rh123, DiBAC4(3), DAPI and acridine orange. Chemunex, the manufacturer of the ChemScan analyzer system, also makes a fluorogenic probe, Chemchrome B, which will be incorporated into the suite of probes to evaluate once their system is on site. Regardless of the combination of stains and probes all will be evaluated on membrane filters.
2. Development of a FISH protocol that will be applicable to our conditions will be continued. Complimentary 16s rRNA probes to *Ps. aeruginosa* and currently in our laboratory will be evaluated first. Once this protocol has been adequately optimized other probes will be ordered for a select number of other species.
3. Currently, protocols to evaluate the effects of disinfection and the resulting lethality, injury on stain and/or probe specificity and reliability are being developed. *E. coli* O157:H7 is the reference strain and chlorine the disinfectant the reference protocol is being developed around. Upon completion of this work, the resulting protocol will be extended to other species and disinfectants (e.g., iodine). Similar disinfectant experiments will then be conducted on the same species after starvation to evaluate the effects of starvation on disinfection resistance and the applicability of the stains and probes.
4. Development of the immunomagnetic separation system will continue. Combined with the

rapid methods described above, with enumeration by the ChemScan, we anticipate that this will provide a highly sensitive technique for the detection of specific, active bacteria.

#### **PRESENTATIONS AND PUBLICATIONS:**

Dr. McFeters was invited to attend the NASA Advanced Technologies Workshop, Enhancement of Human Health and Performance in Space, held at the Center for Advanced Space Studies, Houston, Texas, in September, 1996. He also visited with colleagues at Johnson Space Center.

In November, 1996, Dr. McFeters will present a paper at the American Water Works Association Water Quality Technology meeting in Boston. This paper, entitled "Detection of Specific Respiring Bacteria in Water Using Immunomagnetic Separation Combined with Cyanoditoyl Tetrazolium Chloride", includes data which shows that immunomagnetic separation can be an effective way of concentrating bacteria such as *E. coli* O157:H7 from water and other fluids including food suspensions. Another presentation, "Effect of Chlorine-Induced Injury on Reaction Kinetics of Coliforms and *E. coli* in Colisure and LTB" will be given by Dr. McFeters.

An abstract has been submitted for the 27th International Conference on Environmental Systems, to be held at Lake Tahoe in July 1997 (copy attached). Entitled "Rapid Detection of Bacteria in Spacecraft Water Systems", this presentation will describe and discuss our research in this area.

We are preparing an abstract to be submitted for the American Society for Microbiology Annual Meeting to be held in Miami Beach next May, on rapid, direct microscopic methods for enumeration of specific, active bacteria.

#### **TRAVEL PLANS:**

During his visit to Boston for the AWWA WQTC meeting in November, Dr. McFeters will also meet with Millipore Corporation to discuss research utilizing their new Rapid Micro Detection System. That company is providing us with an instrument to include in our methods development program.

Dr. Pyle and Dr. Lisle will travel to Paris, France, in December for training in the installation and use of the ChemScan instrument by Chemunex.

Dr. McFeters and at least one other team member will attend the American Society for Microbiology Annual Meeting at Miami Beach next May to present research results and confer with colleagues who attend that meeting.

**FY96 DATA UPDATE FORM**  
**LIFE AND BIOMEDICAL SCIENCES AND APPLICATIONS DIVISION**  
**PI INDEX: TASK DESCRIPTION/BIBLIOGRAPHY DATABASE**

*DIRECTIONS: Please provide or update the information contained on the form below. Please do not leave any fields blank.*

**1. COMPLETE TASK TITLE**

Rapid Bacterial Testing for Spacecraft Water

**2. PRINCIPAL INVESTIGATOR**

Last Name: McFeters

First Name or Initial: Gordon

Middle Name or Initial: A.

Prefix Title (Mr., Dr., Prof., etc.): Dr.

Suffix Title (Ph.D., Sc.D., M.D., etc.): Ph.D.

Affiliation: Montana State University

**3. INVESTIGATOR CONTACT INFORMATION**

Telephone Number (Area Code, Number, Extension): 406-994-5663

FAX Number (Area Code, Number): 406-994-4926

E-mail/Internet Address: umbgm@msu.os~~es~~s.montana.edu

Complete Mailing Address:

Department  
Mail Code (if any)  
Institution  
Street  
City, State, Zip Code

Dr. Gordon A. McFeters, Ph.D.  
Department of Microbiology  
Montana State University  
Bozeman, MT 59717

Congressional District:

1

**4. CO-INVESTIGATOR INFORMATION**

Co-Investigator Name(s) and Degree(s)

Co-Investigator Affiliation(s)

1. Barry H. Pyle, Ph.D.

Montana State University

2.

3.

4.

5.

6.

FY96 DATA UPDATE FORM  
LIFE AND BIOMEDICAL SCIENCES AND APPLICATIONS DIVISION  
PI INDEX: TASK DESCRIPTION/BIBLIOGRAPHY DATABASE

Gordon A. McFeters; Montana State University

**5. TASK INFORMATION**

Solicitation (NRA, AO, e.g., 93-OLMSA-07): 95-OLMSA-01  
Task Type (Flight/Ground): Ground  
Task Identification Number: 199-04-17-18  
Grant Number: NAGW-5001  
Joint Agency Participation (NIH, NIST, DoD, etc.): \_\_\_\_\_

**Discipline Name** Please choose ONE (and only one) of the disciplines listed below that best describes this task.

- |   |   |
|---|---|
| <input type="checkbox"/> Cardiopulmonary<br><input type="checkbox"/> Hematology/Immunology<br><input type="checkbox"/> Endocrinology/Metabolism<br><input type="checkbox"/> Pharmacology<br><input type="checkbox"/> Musculoskeletal/Connective Tissue<br><input type="checkbox"/> Neuroscience<br><input type="checkbox"/> Toxicology<br><input type="checkbox"/> Barophysiology<br><input checked="" type="checkbox"/> Microbiology<br><input type="checkbox"/> Advanced Hormone, pH, or Electrolyte Sensors<br><input type="checkbox"/> Methods for Storing Biological Samples<br><input type="checkbox"/> Advanced Displays and Controls Dev.<br><input type="checkbox"/> Human-Machine Function Allocation<br><input type="checkbox"/> Interaction Among Intelligent Agents<br><input type="checkbox"/> IVA & EVA<br><input type="checkbox"/> Analog Studies | <input type="checkbox"/> Situational Awareness<br><input type="checkbox"/> Human Communication<br><input type="checkbox"/> Human Engineering Methodologies<br><input type="checkbox"/> Space Workstations<br><input type="checkbox"/> Telescience, Training, and Maintenance<br><input type="checkbox"/> Strength Decrements<br><input type="checkbox"/> Air Revitalization<br><input type="checkbox"/> Water Recovery<br><input type="checkbox"/> Solid Waste Processing<br><input type="checkbox"/> Plant Production<br><input type="checkbox"/> Food Processing and Storage<br><input type="checkbox"/> Thermal Control Systems<br><input type="checkbox"/> Monitoring & Control<br><input type="checkbox"/> EVA<br><input type="checkbox"/> Technology to Improve EVA Garments<br><input type="checkbox"/> Environmental Monitors and Sensors |
|---|---|

**6. FUNDING**

Period of Performance from (mo/yr): 1/96 to (mo/yr): 12/96  
Yearly Funding (FY1996): \$ 94,923

**Students Affiliated with Task:**

Level	Number
Pre-college	_____
Undergraduate	<u>2</u>
Graduate	_____
Post Docs	<u>1</u>
<b>TOTALS</b>	<u>3</u>

**7. FLIGHT INFORMATION (FLIGHT INVESTIGATORS ONLY)**

Monitoring NASA Center (HQ, ARC, JSC, KSC): HQ  
Flight Hardware (BRIC, AEM, STL, etc.): \_\_\_\_\_  
Current Flight Assignment (STS-89-Neurolab, etc.): \_\_\_\_\_  
Reflight History (STS-9, STS-54, etc.): \_\_\_\_\_

FY96 DATA UPDATE FORM  
LIFE AND BIOMEDICAL SCIENCES AND APPLICATIONS DIVISION  
PI INDEX: TASK DESCRIPTION/BIBLIOGRAPHY DATABASE

FY96 TASK ABSTRACT

Dr. Gordon A. McFeters, Ph.D.

199-04-17-18

Ground

Rapid Bacterial Testing for Spacecraft Water

---

*Directions: Update to reflect any changes in the direction of the task. However, please limit the length of the text. Significant alterations, additions, or deletions should preferably be submitted in both hard copy and electronic form.*

---

In water microbiology, there is a need for rapid methods to enumerate specific viable bacteria. This is a particular concern in relation to water which will be reclaimed for potable use on Space Station Alpha. Our principal objective is to develop procedures which will permit the detection of specific marker bacteria which would be used to monitor the performance of the water reclamation and storage systems. In addition, the techniques will be applicable to the detection of particular pathogenic bacteria. Our novel approach (Pat. Pending), which utilizes membrane filtration and combines a fluorochrome for assessment of respiratory activity with specific fluorescent antibody detection of waterborne bacteria, will be evaluated in comparison with molecular methods which will be developed in this project. These include fluorescent in situ hybridization following membrane filtration and microcolony formation, to permit rapid quantitation of specific, viable bacteria. Fluorescent in situ PCR will also be investigated for sensitive detection of specific bacteria. Results will be applicable not only to Spacecraft systems but will also have applications for earth-based situations. Similar methodologies would be of great value for the examination of clinical and fecal specimens, potable waters, natural waters, foods, and soils, for more timely and reliable detection of specific microbial contamination. Other applications include the examination of purified waters used in the pharmaceutical industry, laboratories and the electronic industry. □ We will develop analytical procedures to identify and quantify bacteria in waste water and product water on spacecraft, permitting more timely measurement and control of bacterial contaminants, and facilitating development of standards and countermeasures to optimize crew health, safety and productivity.



FY96 DATA UPDATE FORM  
LIFE AND BIOMEDICAL SCIENCES AND APPLICATIONS DIVISION  
PI INDEX: TASK DESCRIPTION/BIBLIOGRAPHY DATABASE  
FY96 TASK PROGRESS

Dr. Gordon A. McFeters, Ph.D.

199-04-17-18

Ground

Rapid Bacterial Testing for Spacecraft Water

---

*Directions: Task progress should include a brief but meaningful paragraph about the status of the task. Answers to the following questions should help to formulate an appropriate statement of progress: What has been accomplished thus far? What questions have been answered? What new questions have arisen? How does this year's progress affect future work on this task? Please note that the taskbook is tied to the fiscal cycle, which may not coincide with the funding cycle of individual tasks. However, even tasks for which funding began in the middle of FY96 should report progress for the partial year. Please submit this text in both hard copy and electronic form.*

---

Efforts have been directed towards the development of a technique that allows the staining of bacteria retained on black polycarbonate filters with two or more fluorochromes to determine, from a single filter: (a) the total number of bacteria present in a water sample; (b) if a specific strain of bacteria is present within the total population; and, (c) if these bacteria are viable. The different stains or probes used include: (1) total cell counts using DAPI and acridine orange, (2) identification of specific strains of bacteria using TRITC and FITC labeled secondary antibodies raised against anti-*E. coli* O157:H7 primary antibodies, (3) detection of respiratory activity using CTC, (4) detection of nutrient responsiveness using the direct viable count (DVC) technique, (5) detection of membrane potential using calcofluor white, DiOC<sub>6</sub>(3) and Rh123, (6) detection of loss of membrane potential using DiBAC<sub>4</sub>(3) and (7) determination of viability using the commercially available Live/Dead BacLight kit (Molecular Probes, Inc., Eugene, OR). In addition to these assays, agar plate assays for determination of total viable counts (R2A agar) and lethality and injury within populations of bacteria after exposure to disinfection (TLY and TLYD agar) have been incorporated.

A modified version of the CTC/fluorescent antibody filter assay has been used with *E. coli* O157:H7 cells. Bacteria retained on black polycarbonate filters were incubated with CTC, stained with TRITC labeled fluorescent antibodies, followed by staining with DiBAC<sub>4</sub>(3). The modified protocol produced promising results. In addition, highly efficient (>90%) bacterial detection by immunomagnetic separation (IMS) and the compatibility of IMS with cyanoditolyl tetrazolium chloride (CTC) incubation to determine respiratory activity, using *E. coli* O157:H7 has been achieved. Staining with DAPI or a specific fluorescein-conjugated anti-O157 antibody was used to allow visualization of bacteria by epifluorescence microscopy. TRITC labeled fluorescent antibodies, CTC, DiBAC<sub>4</sub>(3) and the Live/Dead BacLight kit were tested using *E. coli* O157:H7 cultures after exposure to lethal temperatures and concentrations of formalin, ethanol and chlorine. All stains and probes evaluated demonstrated consistent and predictable results with disinfected and starved bacteria. Sub-lethal chlorine injury is being evaluated using the same stains and probes listed above, with the addition of Rh123. Injury is also being determined by use of TLY and TLYD plate agars, where TLYD plates contain deoxycholate which inhibits the growth of injured bacteria.

A ChemScan analysis system (Chemunex, France) has been acquired using Department of Defence (Army) funding. This system includes a dual channel laser that can excite two different fluorochromes or probes simultaneously. It can scan an entire filter (25mm diam.) in less than three minutes, locating and counting cells of specific size and fluorescence (i.e., color). A computerized mechanical microscope stage is used to confirm positive cells.

FY96 DATA UPDATE FORM  
LIFE AND BIOMEDICAL SCIENCES AND APPLICATIONS DIVISION  
PI INDEX: TASK DESCRIPTION/BIBLIOGRAPHY DATABASE  
FY96 EARTH BENEFITS

Dr. Gordon A. McFeters, Ph.D.

199-04-17-18

Ground

Rapid Bacterial Testing for Spacecraft Water

---

*Directions: Answers to the following questions should help to formulate an appropriate statement of Earth benefits: Does this research seek to understand a disease or malady that affects humans on Earth and/or in space? Does this research seek to develop new therapeutics or protocols for alleviating symptoms of a disease or malady on Earth? Will this research yield a new understanding of basic biological processes? What relationship does this task posit between processes on Earth and in space? What impact could the results of this research have on the common man? What benefits are foreseen by the development of this new technology? Please submit this text in both hard copy and electronic form.*

---

The techniques we are developing for rapid detection of specific bacteria in conjunction with viability assessment have attracted significant attention among environmental microbiologists. The combination of immunomagnetic separation with the CTC respiration assay and fluorescent antibody staining permits direct detection of bacterial contaminants within 6-7 hours. Most other methods employ a 12-24 hour enrichment prior to identification. A patent application on this technology is currently being evaluated. One of the impediments to timely assessment of water and food quality has been the time required to obtain results using traditional or even novel techniques. These procedures will be used for monitoring potable water, foods, and parenteral (injectible) liquids.

Our experiments with disinfection and starvation, using these new methods to detect bacteria, will generate more reliable data on bacterial injury, lethality, and survival.

FY96 DATA UPDATE FORM  
LIFE AND BIOMEDICAL SCIENCES AND APPLICATIONS DIVISION  
PI INDEX: TASK DESCRIPTION/BIBLIOGRAPHY DATABASE

FY96 BIBLIOGRAPHY

Dr. Gordon A. McFeters, Ph.D.

199-04-17-18

Ground

Rapid Bacterial Testing for Spacecraft Water

---

*Directions: The list of publications, presentations, and other accomplishments should include all awards and published material, including journal articles, books and book chapters, published meeting papers and abstracts, NASA technical documents, and patents. Please provide complete citation source information for the bibliographic data. In addition, please send a complete reprint or photocopy of each publication. For books and book chapters, please provide the title and copyright pages as well. Include ONLY materials published during FY 1996 (October 1, 1995 to September 30, 1996).*

---

**Huang, C.-T., G.A. McFeters, and P.S. Stewart. 1996.** Evaluation of physiological staining, cryoembedding, and autofluorescence quenching techniques on fouling biofilms. *Biofouling* 9:269-277.

**Lisle, J.T., B.H. Pyle, and G.A. McFeters. 1996.** Evaluation of physiological activity in *E. coli* O157:H7 after disinfection. Accepted for presentation at the 1997 General Meeting of the American Society for Microbiology.

**McFeters, G.A., B. H. Pyle, and S. C. Broadaway. 1996.** Detection of specific respiring bacteria in water using immunomagnetic separation combined with cyanoditotyl tetrazolium chloride. American Water Works Association Water Quality Technology Conference, Boston, MA.

**Pyle, B.H., and G.A. McFeters. 1996.** Detection of respiring *E. coli* O157:H7 using immunomagnetic separation, cyanoditotyl tetrazolium chloride, and a fluorescent antibody. American Society for Microbiology 96th General Meeting, New Orleans, LA, Abstract Q-439, p. 462.

**Pyle, B.H., J.T. Lisle, S.C. Broadaway, and G.A. McFeters. 1996.** Rapid assessment of drinking water quality using novel analytical approaches. Submitted for presentation at the 1997 American Water Works Association Water Quality Technology Conference.

**Pyle, B.H., S.C. Broadaway, AND G.A. McFeters. 1996.** Rapid Enumeration of Respiring *Escherichia coli* O157:H7 in Water and Meat Using Immunomagnetic Separation. Accepted for presentation at the 1997 General Meeting of the American Society for Microbiology.

**Smith, J.J., and G.A. McFeters. 1996.** Effects of substrates and phosphate on INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride) and CTC (5-cyano-2,3-ditotyl tetrazolium chloride) reduction in *Escherichia coli*. *J. Appl. Bacteriol.* 80:209-215.

**Wentland, E.J., P.S. Stewart, C.-T. Huang, and G.A. McFeters. 1996.** Spatial variations in growth rate within *Klebsiella pneumoniae* colonies and biofilm. *Biotechnol. Prog.* 12:316-321.

**FY 1997 Task Book Electronic Data Update Form**  
**NASA Office of Life and Microgravity Sciences and Applications**  
**Life Sciences Division**

PLEASE CAREFULLY READ THE DIRECTIONS FOR EACH SECTION / SUBSECTION OF THE FORM.

ENTER ONLY INTO THE DATA ENTRY BOXES (INDICATED BY SOLID BLACK BORDERS).

PRINT THIS FILE WHEN FINISHED AND RETURN THE HARD-COPY PRINT OUT IN ADDITION TO THE ELECTRONIC FORM.

PLEASE NOTE THAT ALL REVISIONS TO THIS FILE WILL BE CLEARLY MARKED ON THE PRINTED COPY FOR DATA ENTRY PURPOSES. PLEASE DO NOT TURN OFF THIS FEATURE.

SAVE THIS FILE (SAME FILE NAME) AS A WORD 6.0 FILE WHEN FINISHED.

PLEASE MAKE SURE TO ENTER "Yes" IN THE ENTRY BOX BELOW IN ORDER TO INFORM US THAT THE FORM HAS BEEN REVIEWED.

NOTE: ITALICS, SUPERSCRIPTS, SUBSCRIPTS, AND OTHER SPECIAL CHARACTERS PRESENT IN THE ORIGINAL DATA BASE COULD NOT BE TRANSFERRED TO THIS ELECTRONIC FORM. YOU ARE NOT REQUIRED TO RESTORE THESE CHARACTERS IN THIS FILE. THESE SPECIAL CHARACTERS WILL BE PRESERVED IN THE PUBLISHED FY97 TASK BOOK. THE HARD-COPY VERSION OF THIS FORM (SENT TO YOU VIA MAIL) DOES DISPLAY THESE CHARACTERS AND, THEREFORE, MAY BE USED AS A REFERENCE.

Has this file been reviewed and updated?

<u>Yes</u>
No

# SECTION I. Task and Investigator Information

**DIRECTIONS:** Starting with "Task Title" Box, proceed through the data entry boxes (indicated by solid black borders). Please provide or update all applicable information.

## A. Task Info.

Task Title:

**Rapid Bacterial Testing for Spacecraft Water**

Project Type:

**Ground**

Flight Program:

Flight Hardware:

## B. Principal Investigator

Last Name:

**McFeters**

First Name or Initial:

**Gordon**

Middle Name or Initial:

**A.**

Prefix Title (Mr., Ms., Dr., Prof., etc.):

**Dr.**

Suffix Title (Ph.D., Sc.D., M.D., etc.):

**Ph.D.**

Affiliation:

**Montana State University**

Address:

**Department of Microbiology  
Montana State University  
Bozeman, MT 59717**

Phone:

**406-994-5663**

Fax:

**406-994-4926**

Email:

**umbgm@msu.oscs.montana.edu**

**C. Additional Investigators****Co-Investigators:**

**NOTE:** Please enter/edit Co-Investigator information in the following format:

First Name\_Middle Initial\_Last Name,\_Suffix;\_Affiliation

i.e., John A. Doe, Ph.D.; University of Anywhere

1.	<b>Dr. Barry H. Pyle, Ph.D.; Montana State University</b>
2.	<b>Dr. John T. Lisle, Ph.D.; Montana State University</b>
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	
11.	
12.	

**Number of Funded Students:**

Pre College:

Undergraduate:

**2**

Graduate:

Post Doctoral:

**1**

## SECTION II. Task Description

**DIRECTIONS:** The Task Book Task Description consists of 3 concatenated components: Abstract, Task Progress, and Earth Benefits (in that order). Each should be entered into the data entry boxes provided (boxes will expand as necessary). Please consider the final concatenation when creating/editing these documents in order to ensure a logical flow of thought.

### A. Abstract

**DIRECTIONS:** The provided abstract has been taken from either the FY96 Task Book or the original project proposal (if the task was not in last year's Task Book). Please update to reflect any changes in the direction of the task. If we were unable to locate an abstract, please provide one.

In water microbiology, there is a need for rapid methods to enumerate specific viable bacteria. This is a particular concern in relation to water which will be reclaimed for potable use on Space Station Alpha. Our principal objective is to develop procedures which will permit the detection of specific marker bacteria which would be used to monitor the performance of the water reclamation and storage systems. In addition, the techniques will be applicable to the detection of particular pathogenic bacteria. Our novel approach (patent pending), which utilizes membrane filtration and combines a fluorochrome for assessment of respiratory activity with specific fluorescent antibody detection of waterborne bacteria, will be evaluated in comparison with molecular methods which will be developed in this project. These include fluorescent in situ hybridization following membrane filtration and microcolony formation, to permit rapid quantitation of specific, viable bacteria. Fluorescent in situ PCR will also be investigated for sensitive detection of specific bacteria. Results will be applicable not only to spacecraft systems but will also have applications for Earth-based situations. Similar methodologies would be of great value for the examination of clinical and fecal specimens, potable waters, natural waters, foods, and soils, for more timely and reliable detection of specific microbial contamination. Other applications include the examination of purified waters used in the pharmaceutical industry, laboratories, and the electronics industry.

Experiments were performed using a suite of fluorescent stains that demonstrate a range of cellular physiological activities and properties that are related to viability. These stains were applied to the pathogen *Escherichia coli* O157:H7 exposed to low levels of disinfection with chlorine. Freshly prepared suspensions and starved cultures were examined. With earlier funding, we developed a rapid method for detecting specific respiring bacteria. A prototype system has been demonstrated with *Escherichia coli* O157:H7 suspensions and inoculated

ground beef. In FY97, we have continued evaluation of this hybrid technique, which includes immunomagnetic separation, and incubation with cyanoditoyl tetrazolium chloride (CTC) to determine respiratory activity, followed by fluorescent antibody staining (IMS/CTC/FAb).

Our objective is to

~~We will develop~~ analytical procedures to identify and quantify bacteria in waste water and product water on spacecraft, permitting more timely measurement and control of bacterial contaminants, and facilitating development of standards and countermeasures to optimize crew health, safety, and productivity.



## **B. Task Progress**

**DIRECTIONS:** Task Progress must reflect any progress made during FY97 (Oct. 1, 1996 to Sept. 30, 1997) even if the project was active for only part of this time period. If this task was included in the FY96 Task Book, **PLEASE DO NOT DUPLICATE THE FY96 TASK PROGRESS**. If you wish to refer to last year's Task Progress as a guide, you can access the online Task Book Data Base (via <http://peer1.idi.usra.edu>) or contact John Nelson at 202-488-5131 (or [john.nelson@hq.nasa.gov](mailto:john.nelson@hq.nasa.gov)) for a copy. Answers to the following questions should help to formulate an appropriate statement of progress: What has been accomplished thus far (and particularly during FY97)? What questions have been answered? What new questions have arisen? How does this fiscal year's progress affect future work on this task?

We have used a suite of stains and probes, in conjunction with viable plate counts, to assess the effect of chlorine disinfection on membrane potential (Rhodamine 123 [Rh123] and DiBAC<sub>4</sub>3), membrane integrity (BacLight Live/Dead Kit, Molecular Probes Inc.), respiratory activity (CTC), and substrate responsiveness (direct viable count [DVC]) in *E. coli* O157:H7. After a 5 minute exposure to chlorine, physiological indices were affected in the following order: viable plate count > substrate responsiveness > membrane potential > respiratory activity > membrane integrity. In situ assessment of physiological activity using a multi-phasic approach, as demonstrated in this study, permits more comprehensive decisions to be made in regard to determining the site and extent of injury in bacterial cells.

In related experiments, cultures of *E. coli* O157:H7 were starved in M9 minimal medium with no added carbon source, for 14 days at 21-23°C. In addition to the assays listed above, intracellular esterase activity was determined using Fluorassure (Chemunex, France) in which case bacteria on filters were enumerated with a Scan RDI (Chemunex) rapid laser scanner. Assays were performed to demonstrate the influence of starvation on susceptibility to disinfection with 0.5 ppm chlorine. Results indicated that between inoculation and starvation through day 5, the assays used demonstrated an increased number of physiologically active cells, with numbers remaining relatively constant through day 14. However, cells exhibiting membrane potential and substrate responsiveness declined as starvation progressed, while resistance to chlorine disinfection increased as indicated by the percentage of injured cells. Injury decreased from 92.6% of cells at time 0, to 44.3% by day 5, and remained relatively constant through day 14 (35.3%). The results show that in the starvation conditions used, this strain of *E. coli* maintained physiological activity while increasing resistance to chlorination.

For the IMS/CTC/FAb evaluation, we have added the more rapid and sensitive Scan RDI system. Using the IMS/CTC/FAb procedure, we recovered more than 86% of the O157 cells in

the inoculum, with regression coefficients >0.95 when comparing the CTC/FAb counts with those obtained by plate count enumeration of cells recovered by immunomagnetic beads. Epifluorescence microscopy has a lower detection limit of ca.  $10^3$  cells per g or per ml of sample, while the Scan RDI system permits detection of 1 cell per g or per ml.

The results of experiments conducted in this period demonstrate the utility and versatility of the novel rapid analytical methodology under investigation. Although only one of the present suite of physiological fluorochromes as well as the IMS/CTC/FAb were compatible with the current Scan RDI instrumentation, our results thus far provide justification for optimism concerning the prospect of applying this powerful new rapid approach to bacterial detection in a wide range of settings. Discussions with the manufacturer of the Scan RDI system continue to focus on avenues for improving the versatility and cost of this instrument as it is reconfigured. In addition, we will continue to evaluate and validate additional physiological fluorochromes for this kind of instrumentation, based on our experience with stains that assess specific physiological targets. Ultimately, this line of investigation will lead to more accurate methods for bacterial identification and viability determination associated with this rapid detection strategy.

### **C. Earth Benefits**

**DIRECTIONS:** Please update the Earth Benefits for this project as necessary. If this task was not included in last year's Task Book, please provide a statement of Earth Benefits. Answers to the following questions should help to formulate an appropriate statement of Earth Benefits: Does this research seek to understand a disease or malady that affects humans on Earth and/or in space? Does this research seek to develop new therapeutics or protocols for alleviating symptoms of a disease or malady on Earth? Will this research yield a new understanding of basic biological processes? What relationship does this task posit between processes on Earth and in space? What impact could the results of this research have on the human race? What benefits are foreseen by the development of this new technology? \_

The techniques we are developing for rapid detection of specific bacteria in conjunction with viability assessment have attracted significant attention among environmental microbiologists. The combination of immunomagnetic separation with the CTC respiration assay and fluorescent antibody staining permits direct detection of bacterial contaminants within six-seven hours. Most other methods employ a 12-24 hour enrichment prior to identification. A patent application on this technology is currently being evaluated. One of the impediments to timely assessment of water and food quality has been the time required to obtain results using traditional or even novel techniques. These procedures will be used for monitoring potable water, foods, and parenteral (injectible) liquids.

Our experiments with disinfection and starvation, using these new methods to detect bacteria, will generate more reliable data on bacterial injury, lethality, and survival. The combination of methodological approaches for bacterial concentration, detection and viability assessment being investifated has significant benefits both immediately and in the long-term. For example, our present target bacterium (*E. coli* O157:H7) is currently a persistent and significant food borne health threat in the U.S. Other aspects of developing this technology hav been funded by the U.S. Department of Defense (Army) and the National Institutes of Health (Environmental Health Institute). A patent is pending, and we have partnered with Montana ImmunTech, Inc., a local biotechnology development company to bring this technique to the U.S. and international market place. Ultimately, the development of novel antibodies may lead to therapeutics and possibly vaccines for the treatment of a range of enteric diseases which are caused by bacteria.

It is anticipated that our new methodological approach will be applied in studies by others to determine the ecology of *E. coli* O157 and related bacteria in the food source animal population and their environment and, as a consequence, assist in its reduction or elimination.

Some version of this technology might also be amenable to miniaturization and use in protecting crew health on space vehicles. It is also applicable to the rapid detection of other target bacteria in a range of contexts including industrial, clinical and environmental settings.

## SECTION III. FY97 Bibliography

**DIRECTIONS:** Please provide complete citation source information for publications, presentations, and other accomplishments resulting from this task DURING FY97 ONLY (Oct. 1, 1996 to Sept. 30, 1997). In addition, please send a complete reprint or photocopy of each publication. If there are no FY97 bibliographic entries to be included, please enter "NONE" into the shaded box below.

DO include:

- articles and abstracts published in FY97 in peer-reviewed journals.
- meeting papers or abstracts published in FY97.
- academic dissertations and theses published in FY97.
- books and book chapters published in FY97.
- NASA technical documents published in FY97.
- any of the above accepted for publication in FY97 (label as "In Press").
- presentations in FY97 without publications (label as "Presentation").
- new patents in FY97 (label as "In Process", "Pending", or "Approved").

DO NOT include:

- anything published or presented before 10/1/96.
- manuscripts NOT YET ACCEPTED during FY97 for publication.
- required NASA reports.
- related grant awards.
- presentations - IF a corresponding publication has already been cited.

Please enter bibliographic entries into the entry box below.

Please use the following author name format:

Primarylastname, F.M., Secondarylastname, F.M., and Lastlastname, F.M.

Lisle, J.T., Pyle, B.H., and McFeters, G.A. 1997. Evaluation of physiological activity in *E. coli* O157:H7 after disinfection. American Society for Microbiology, 97<sup>th</sup> General Meeting, Miami Beach, Florida, May 4-8, 1997, Abstracts, p. 455, Q-3.

Pyle, B.H., Broadaway, S.C., and McFeters, G.A. 1997. Rapid enumeration of respiring *Escherichia coli* O157:H7 in water and meat using immunomagnetic separation. American Society for Microbiology, 97<sup>th</sup> General Meeting, Miami Beach, Florida, May 4-8, 1997, Abstracts, p. 480-480, Q-151.

Pyle, B.H., Lisle J.T., Broadaway, S.C., and McFeters, G.A. 1997. Rapid detection of bacteria

in spacecraft water systems. SAE Technical Paper Series No. 972421. 27<sup>th</sup> International Conference on Environmental Systems, Lake Tahoe, Nevada, July 14-17, 1997. Society for Automotive Engineers.